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Relationship Between Molecular Configuration and Tensile Properties of Protein Fibers*

G. C. Nutting, M. Halwer, M. J. Copley, and F. R. Senti

Eastern Regional Research Laboratory,† Philadelphia

IT has been demonstrated that globular proteins can be changed into the extended, quasi-crystalline molecular form typical of natural fibrous proteins and that the change produces a notable increase in fiber tenacity [1, 2, 3]. To show more fully the effect of altered molecular configuration on mechanical properties of protein fibers, data are presented in this paper on stress-strain characteristics—strength, elongation, stiffness, toughness, and elasticity—of one globular protein (ovalbumin) and the fibrous proteins keratin, silk fibroin,‡ and collagen, in corresponding configurational states. Ovalbumin was chosen as the most suitable globular protein, because of the especial ease and completeness with which its molecules can be given a linear configuration. Comparison of its tensile properties with those of keratin was particularly sought since diffraction patterns of oriented globular proteins and stretched (*beta-*) keratin prove the near identity of their spatial arrangement. Tensile tests were made on keratin in its normal, somewhat folded (*alpha-*) configuration; in its fully extended, *beta*-keratin form; and after supercontraction as disoriented keratin. Collagen and fibroin filaments were tested in the ordinary, fibrous condition, and also after disorientation by contraction.

Materials and Experimental Procedures

Ovalbumin, prepared from the whites of fresh eggs by the method of Sørenson and Høyrup [4], was mixed with half its weight of distilled water, kneaded into a plastic mass, extruded through a 13 mil die, and denatured by boiling ½ hour in distilled water. Orientation was achieved by stretching the denatured filaments in steam. The stretched fibers were relaxed by immersing several hours in water at 25°C. No formaldehyde or other chemical after-treatment was given. Fiber diameters ranged from 150 to 400 microns.

Wool, mohair, and horsehair* were the keratin fibers used. The wool was a high-grade 62s blend, received in the form of top. The mohair ranged in diameter from 23 to 34 microns. The horsehair used for most of the tests was black, with diameter 90 to 170 microns, averaging 125. White, violin-bow horsehair was generally unsuitable, since most of the filaments had a low cystine content and contracted spontaneously in boiling water, owing probably to

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† One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, United States Department of Agriculture.

‡ Hereafter the term "fibroin" will be used.

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photochemical damage while still on the animal. Selected, undamaged hairs, however, were used for the load-elongation experiments reported below, the black hair being too short for this purpose. All the keratin samples were washed successively in alcohol, ether, and water.

The collagen was U.S.P. size 5–0 surgical gut. It consisted of a single strand with a twist of two turns per inch. It had received no hardening or tanning treatment but had been stretched during manufacture.

The fibroin was U.S.P. size 000 suture silk. Each strand consisted of three plies twisted together, each ply being composed of about 100 filaments 10 microns in diameter. For these tests the strands were separated into their component plies.

Horsehair was converted into the *beta*-keratin form by stretching to relative length * (r.l.) 1.70 in steam and "set" by steaming 3 hours in the stretched state to stabilize it against water at room temperature. After the fibers had been cut out of the stretching clamp, they were relaxed by soaking for several hours in water at room temperature. During relaxation, the fibers contracted until the final r.l. was 1.63. To determine the effect of steaming in the stretched state, horsehair was converted into the *beta*-keratin form without steaming by stretching to r.l. 1.50 in cold water. Since these fibers were not set, it was necessary that they be tested without being relaxed. The results thus obtained gave a measure of the maximum strength obtainable from horsehair in the *beta*-keratin form, since the deleterious effect of steam and the disorientation produced by relaxing were eliminated.

Wool was put into the *beta*-keratin form by mounting small parallelized bundles in clamps and stretching to r.l. 1.50 in cold water and was then set by steaming 3 hours while stretched. The fibers were relaxed in water at room temperature. The final r.l. was 1.41.

Mohair was stretched to r.l. 1.80 in steam and set by steaming one hour. The stretched fibers were relaxed as above. The final r.l. was 1.67. Mohair was selected because its length, uniformity of diameter, and freedom from medullation make it particularly suitable for single-fiber tests. Thus it can be compared with horsehair more readily than can wool, whose nonuniform diameter makes it inapt for single-fiber tests. A comparison between horsehair and mohair was necessary to establish the influence, if any, of fiber diameter on the properties studied.

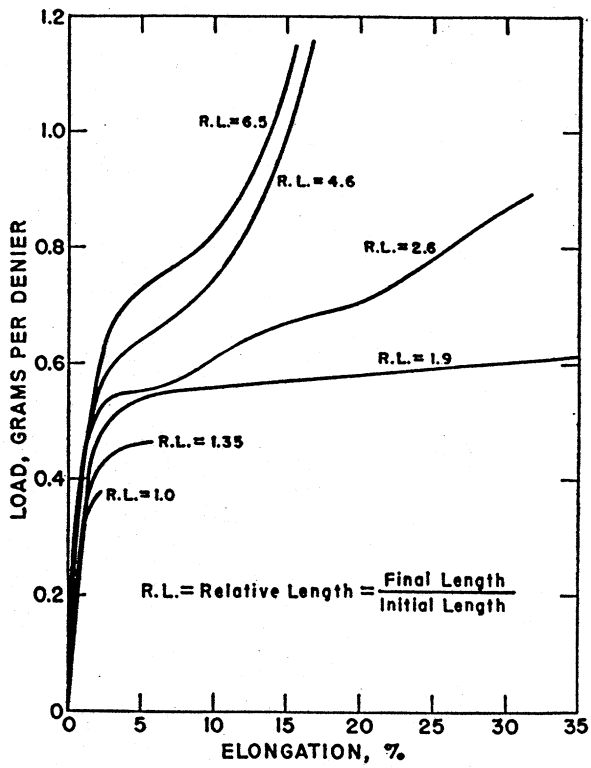

FIG. 1. *Effect of orientation on load-elongation curves of ovalbumin.*

Wool and horsehair were supercontracted by modifications of methods developed by Speakman [5] and Astbury [6]. Wool was supercontracted by stretching parallelized bundles 50 percent in cold water, steaming 3 minutes while stretched, releasing and steaming one hour further. The final r.l. was 0.75. Horsehair was stretched 40 percent in cold water, then boiled 3 minutes in an 0.05*M* phosphate buffer of pH 8.0 (measured at 25°C). The fibers were now released from the clamp, boiled ½ hour in the buffer, washed in distilled water, and dried. The average supercontraction was 21 percent, corresponding to r.l. 0.79. When stretched horsehair was steamed, as was the wool, supercontraction was nonreproducible. The variability was found to be a pH effect and was eliminated by substitution of buffer solution for steam.

Collagen and fibroin were tested in two stages of orientation. The higher stage was that of the ma

* Relative length (r.l.) = final length/initial length. In the experiments described there was usually concurrent change in molecular orientation and fiber length. Sometimes the fiber was stretched and subsequently contracted to less than its unstretched length, making inappropriate such conventional descriptions as "percent stretch" or "draw ratio." To indicate the dimensional changes we have chosen to use the somewhat cumbersome term "relative length." By this is meant the ratio of the final fiber length to the initial length in a particular series of operations.

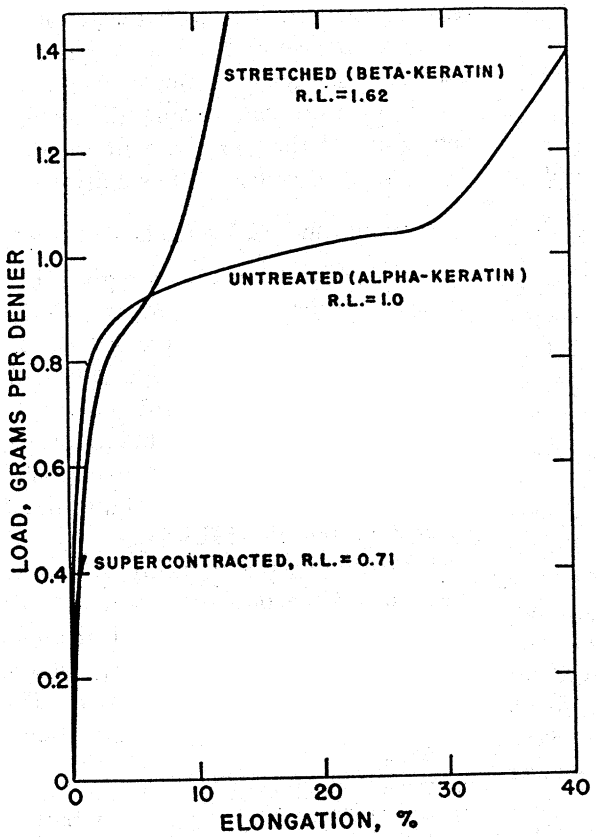

FIG. 2. *Effect of orientation on load-elongation curves of keratin (horsehair).*

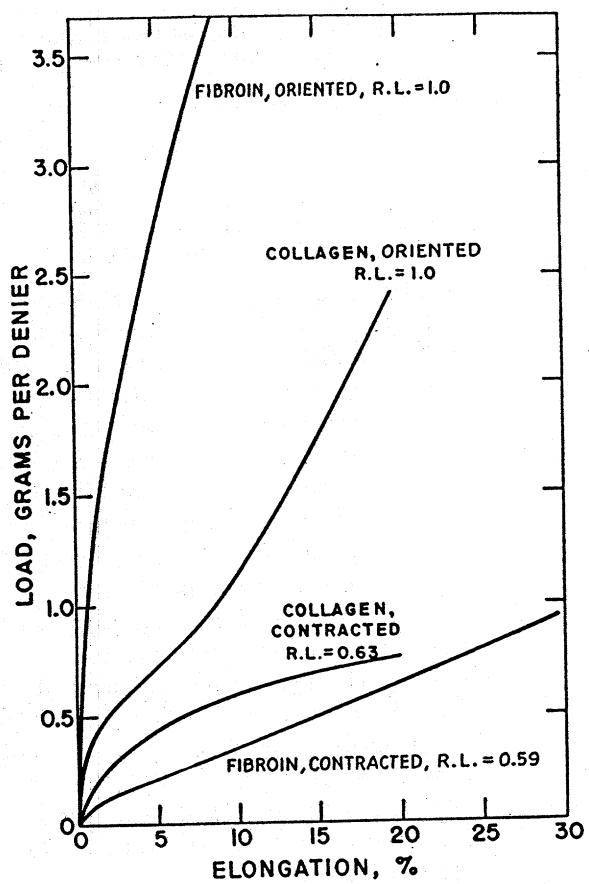

FIG. 3. *Effect of orientation on load-elongation curves of fibroin and collagen.*

terials as received. The samples could be stretched only a little before breaking, except following drastic chemical treatment, and their orientation could not be much improved. A lower stage of orientation was induced by contraction. This was easily done for collagen by immersing the fiber in water at 70°C for 30 seconds, an average contraction to r.l. 0.54 being obtained. Fibroin was contracted to r.l. 0.54 by immersing it for 90 seconds in 8.42 *M* HCl at room temperature. It was washed in water, followed by 1 percent sodium acetate, and again by water. The concentration of acid required to produce contraction was remarkably critical. Thus, 8.00*M* HCl produced no contraction, whereas a concentration of 8.42*M* produced a contraction of 46 percent. Different acids showed different minimum concentrations below which contraction did not occur, but all agreed in the sharpness of the minimum. A more complete account of this phenomenon is in preparation.

In all cases in which a change in orientation was produced as a result of dimensional change, control samples were prepared in which changes in orientation were minimized by keeping the fibers clamped at their original length. In every other respect, the controls received the same treatment as the experimental samples.

To determine to what degree the disorientation process can be reversed, the contracted forms of horsehair and collagen were restretched. Horsehair was contracted to r.l. 0.74, stretched in steam to r.l. 1.78, steamed 3 hours while stretched, then relaxed in water. The final r.l. was 1.68. Thus, the reoriented horsehair was at approximately the same extension as the samples which had been stretched without prior supercontraction. Catgut, contracted to r.l. 0.54, was stretched to r.l. 1.19 in water at 50°C. After drying in the clamps, the fibers were relaxed in the free state to constant length in water at room temperature. Their final r.l. was 0.95.

Appropriate control samples were prepared for the reoriented horsehair, but not for the reoriented collagen. Instead, the same controls which had formerly been prepared for the contracted samples were

TABLE I. Effect of Orientation on Tensile Properties of Ovalbumin Fibers

Relative length	Tenacity (g./den.)	Wet/dry tenacity ratio	Flexibility
1.0	0.60	0.28	0.77
2.4	0.89	0.44	0.81
4.2	1.14	0.54	0.37
5.8	1.44	0.52	0.23

used, on the assumption that a brief exposure to water at 50°C was unlikely to produce appreciable change in fibers which had already been exposed at 70°C.

In all cases, the effectiveness of the various treatments in changing molecular orientation was determined from x-ray diffraction patterns of the product. Diffraction results are discussed below.

Tenacities were measured with a Scott IP–2 testing machine at 70°F and 65 percent R.H. Values reported are the mean of ten tests. Samples were permitted at least 16 hours to reach moisture equilibrium before testing. Deniers of the mohair fibers were determined microscopically and for all other fibers by weighing a measured length. Single fibers of horsehair, mohair, collagen, and ovalbumin were tested. A modified A.S.T.M. bundle test, similar to that of Peterson *et al.* [7], was used for wool. For fibroin, strands consisting of about 100 filaments with a twist of five turns per inch were used. Wet tenacities were determined immediately after soaking the fibers in distilled water at room temperature for one-half to one hour. Knot strengths were measured by tying a simple overhand knot in the fiber and then determining the breaking strength. The ratio of the knot strength to the straight-pull strength is reported as "flexibility." *

Strength and Flexibility Measurements

Results of tenacity tests are given in Tables I and II and may be summarized as follows. For ovalbumin the dry tenacity increased regularly from 0.6 g./den. at r.l. 1 to 1.44 g./den. at r.l. 5.8. (By r.l. 3, incipient resolution of the diffraction pattern into arcs demonstrated partial orientation of peptide chains parallel to the direction of drawing, and at r.l. 6 the *beta*-keratin structure was fully developed.) On stretching, the flexibility appeared to increase at first, then decreased markedly, so that at r.l. 5.8 the knot tenacity was only 0.3 g./den. A similar relation between stretch, tenacity, and flexibility was observed by McMeekin *et al.* [8], who worked with quinone-hardened casein filaments. This is notable because it implies molecular orientation even though

* The knot test gives a measure of the diminution in strength when a fiber is bent over a small mandrel. The test is complex, involving elements of tension, compression, and shear, and is correspondingly difficult to analyze, but, especially when the knot strength is divided by the ordinary tensile strength, it provides a useful index of fiber brittleness.

TABLE II. Effect of Orientation on Tensile Properties of Natural Protein Fibers

Test No.	Material and treatment	Relative length	Tenacity (g./den.)	Wet/dry tenacity ratio	Flexibility
1	Horsehair, untreated (*alpha*)	1.00	1.46	0.87	0.62
2a	Horsehair, stretched, steamed (*beta*)	1.63	1.42	0.65	0.13
2b	Horsehair, control for 2a (*alpha*)	1.00	1.22	0.79	0.62
3a	Horsehair, supercontracted	0.79	0.63	0.44	0.78
3b	Horsehair, control for 3a (*alpha*)	1.00	1.27	0.70	0.58
4a	Horsehair, reoriented (*beta*)	1.68	1.12	0.62	0.33
4b	Horsehair, control for 4a (*alpha*)	1.00	1.15	0.78	0.77
5	Horsehair, stretched, not steamed (*beta*)	1.50	2.06	—	0.27
6	Wool, untreated (*alpha*)	1.00	1.08	0.71	—
7a	Wool, stretched, steamed (*beta*)	1.41	1.08	0.66	—
7b	Wool, control for 7a (*alpha*)	1.00	0.97	0.72	—
8a	Wool, supercontracted	0.75	0.46	0.39	—
8b	Wool, control for 8a (*alpha*)	1.00	1.03	0.70	—
9	Mohair, untreated (*alpha*)	1.00	1.44	—	0.87
10a	Mohair, stretched, steamed (*beta*)	1.67	1.89	—	0.24
10b	Mohair, control for 10a (*alpha*)	1.00	1.32	—	0.87
11	Collagen, oriented	1.00	2.82	0.75	0.48
12a	Collagen, contracted	0.54	1.05	0.60	0.76
12b	Collagen, control for 12a and 13	1.00	2.43	0.82	0.49
13	Collagen, reoriented	0.95	1.77	0.59	0.54
14	Fibroin, oriented	1.00	3.88	0.71	0.79
15a	Fibroin, contracted	0.54	1.44	0.67	1.00
15b	Fibroin, control for 15a	1.00	3.45	0.79	0.93

TABLE III. EFFECT OF ORIENTATION ON MECHANICAL PROPERTIES OF PROTEIN FIBERS, DEDUCED FROM LOAD-ELONGATION CURVES

Material and treatment	Relative length	Ultimate elongation, %	Tenacity, g./den.	Stiffness* g./den.	Toughness index,† g./den.	Elasticity‡ %
Ovalbumin, denatured	1.0	2.2	0.36	17.1	0.004	2.2
Ovalbumin, stretched	1.35	8.4	0.43	5.8	0.02	3.0
Ovalbumin, stretched	1.9	33.8	0.64	2.2	0.11	4.0
Ovalbumin, stretched	2.6	32.6	0.92	2.8	0.15	3.0
Ovalbumin, stretched	4.6	17.8	1.16	6.6	0.11	3.3
Ovalbumin, stretched	6.5	15.3	1.17	7.7	0.09	3.4
Horsehair, supercontracted	0.71	3.5	0.42	16.5	0.008	2.4
Horsehair, untreated (*alpha*)	1.0	40.0	1.40	3.5	0.28	4.1
Horsehair, stretched (*beta*)	1.62	12.9	1.54	11.9	0.10	2.9
Fibroin, contracted	0.59	32.9	0.98	3.0	0.17	—
Fibroin, oriented	1.0	9.1	3.65	40.5	0.17	—
Collagen, contracted	0.63	20.5	0.76	3.9	0.08	—
Collagen, oriented	1.0	21.9	2.66	12.1	0.30	2.6

* Stiffness = T/E, where T = tenacity in g./den. and E = ultimate elongation, expressed as a fraction of the original length.

† Toughness index = $\frac{TE}{2}$.

‡ Elasticity = elongation up to yield point of load-elongation curve.

the spatial order of the casein molecules is not perfect enough to give a fiber diffraction pattern comparable to that of ovalbumin. Wet tenacity of ovalbumin filaments rose with orientation from 0.17 to 0.75 g./den.

The keratins showed remarkably similar behavior. Thus *alpha*-keratin horsehair (1.22 g./den., test 2b, Table II) when stretched and steam-set became *beta*-keratin with strength 1.42 g./den., wet/dry tenacity ratio 0.65, and flexibility 0.13 (test 2a). When the stretched horsehair was contracted to r.l. 0.79 (test 3a), orientation as measured by diffraction was largely removed, the tenacity dropped to 0.63 g./den. and the wet/dry tenacity ratio to 0.44, while flexibility increased to 0.78. Impairment of *alpha*-keratin, produced by a 3-hour steam treatment analogous to that employed to stabilize the *beta*-keratin configuration, amounted to 0.24 g./den. (tests 1 and 2b). The maximum average strength recorded was 2.06 g./den., obtained by stretching horsehair to r.l. 1.5 in water and letting the specimen dry while clamped (test 5). The usual practice of relaxing in cold water could not, of course, be applied. Reorientation of contracted horsehair (test 4a) raised the strength and decreased the flexibility as expected, but the degree of orientation achieved was considerably less than in the initial preparation of *beta*-keratin by stretching *alpha*-keratin. Data on wool (tests 6–8) and mohair (tests 9 and 10), while less complete, show similar effects of orientation on tensile properties. The unexpectedly small increase in tenacity when wool was converted to the *beta*-keratin form (Table II, tests 7a and 7b) might at first be taken to indicate that the rupture of wool under stress is due to slippage of cells rather than to slippage of protein chains. It seems more likely that the considerable difficulty experienced in making bundles for testing from stretched wool was responsible for the low strength.

Oriented collagen, on contraction to r.l. 0.54, underwent a change in tenacity from 2.43 to 1.05 g./den. (tests 12a and 12b). As with keratin, only partial reorientation was attained, with strength rising to 1.77 g./den. at r.l. 0.95 and flexibility diminishing from 0.76 to 0.54. Analogous results on collagenous material have been obtained by Valko, Wöhlisch, and others [9].

The tenacity of fibroin changed from 3.45 to 1.44 g./den. on contraction to r.l. 0.54 in 8.4*M* HCl (tests 15a and 15b). Especially noteworthy was the high initial flexibility, 0.93, since the degree of orientation was evidently somewhat higher than in even the best oriented specimens of collagen, keratin, and ovalbumin.

Load-Elongation Properties

Figure 1 shows typical load-elongation curves for ovalbumin fibers at several stages of orientation, while Figure 2 presents the curves for horsehair, and Figure 3 for collagen and fibroin. In Table III average values for the tenacity and ultimate elongation are listed, together with the stiffness, the toughness in-

dex, and the elasticity.* The last three properties are also derived from the load-elongation curves and have been commended by Smith [10] for the characterization of fibrous materials. An IP–2 testing machine was used to obtain the curves. Owing to the wide range in denier and strength of the various fibers, it was impracticable to keep the rate of loading constant. For horsehair it varied from 1.0 to 2.7 g. per denier per minute; for mohair, 4.9 to 8.0; wool, 1.6 to 1.8; ovalbumin, 0.7 to 1.8; collagen, 1.3 to 3.1; contracted fibroin, 1.6; oriented fibroin, approximately 10. Specimens ranged from 7 to 18 inches in length, depending principally upon the extensibility of the material tested and the capacity of the machine.

Ovalbumin. Shape changes of the ovalbumin curves with orientation are noteworthy. Unoriented fibers (r.l. 1) were incapable of supporting the stress required to produce flow during the test. The extensibility was therefore only a few percent and consisted almost entirely of elastic elongation. A small amount of orientation (r.l. 1.35) strengthened the fiber sufficiently to permit flow, with a resultant significant increase in elongation at break. At r.l. 1.9 elongation was maximal, but there was little reinforcement during the flow process, indicating that the principal units of flow were micelles consisting of large groups of molecules. At r.l. 2.6, elongation was still high and there was distinct reinforcement, which became pronounced at r.l. 4.6. This implies that the chief unit of flow in the latter case was the individual molecule. The molecules straighten out, pack more closely, and interact more strongly among themselves, with consequent reinforcement. At r.l. 6.5 the shape of the curve was much the same as at r.l. 4.6. There was a sharp drop in elongation between r.l. 2.6 and 4.6, corresponding to the change in the flow mechanism and to the partial straightening of chains produced by stretching.

Comparison of Table III with Table I shows that, except for the slightly stretched fibers, there is good correspondence between ultimate elongation and knot-strength flexibility. The large decrease in flexibility between r.l. 2.4 and 4.2 (Table I) was paralleled by the decrease in elongation between r.l. 2.6 and 4.6 (Table III). Flexibility seems to be the more sensitive function of orientation.

Tenacity values in Table III tend to be a little lower than those in Table I, a result to be expected from the greater length of the specimens employed. Both the stiffness, which is related to the pliability of fabric woven from the fiber, and the toughness index, which is a measure of the fiber's capacity to absorb work without breaking, were affected strongly by orientation. At r.l. about 2.5, elongation, flexibility, and toughness were at a maximum, while stiffness was nearly minimal. Tenacity and wet strength were improved by further stretching, but only at the expense of other desirable properties. The (Hooke's law) elasticity was practically independent of the state of orientation.

Keratin (Horsehair). The stress-strain curves for horsehair are similar to those for ovalbumin. Disoriented (supercontracted) horsehair, like unoriented ovalbumin, was too weak to flow and broke after only slight elongation. The normal (*alpha*-) configuration resembled ovalbumin of r.l. 2.6 in having high extensibility, but showed more distinct reinforcement. Stretched horsehair (*beta*-keratin) resembled highly stretched ovalbumin in the general form of its curve and in its sharply diminished elongation at break.

On comparing Table III with Table II (tests 1 and 2a) it is evident that the large decrease in elongation that accompanied *alpha*- to *beta*-keratin conversion was matched by the drop in knot-strength flexibility. The analogy with ovalbumin is clear. The same is true of the contrast between the low elongation of supercontracted keratin and its high flexibility ratio (test 3a, Table II). Like ovalbumin, the elasticity of horsehair did not change much with the state of orientation. It is doubtful whether the differences observed are significant, in view of the difficulty of fixing the yield point exactly. The well-known long-range reversible elasticity to which *alpha*-keratin fibers owe so much of their value was not, of course, manifested in these tests.

Collagen. Oriented collagen gave a curve of the same general form as horsehair and oriented ovalbumin. Contracted collagen, however, showed no reinforcement, from which it appears that the unit of flow was the micelle. Since there was no definite yield point, no estimate of elasticity was made. The elongation of collagen was not much changed by contraction. The increase in elongation which should result through folding of the linear molecules during contraction was offset by the weakening effect of disorientation, causing the fiber to break before the molecules were fully extended. The better oriented form had a much greater toughness index than the contracted form.

* Quantitative definitions of stiffness, toughness, and elasticity are given in Table III (footnotes).

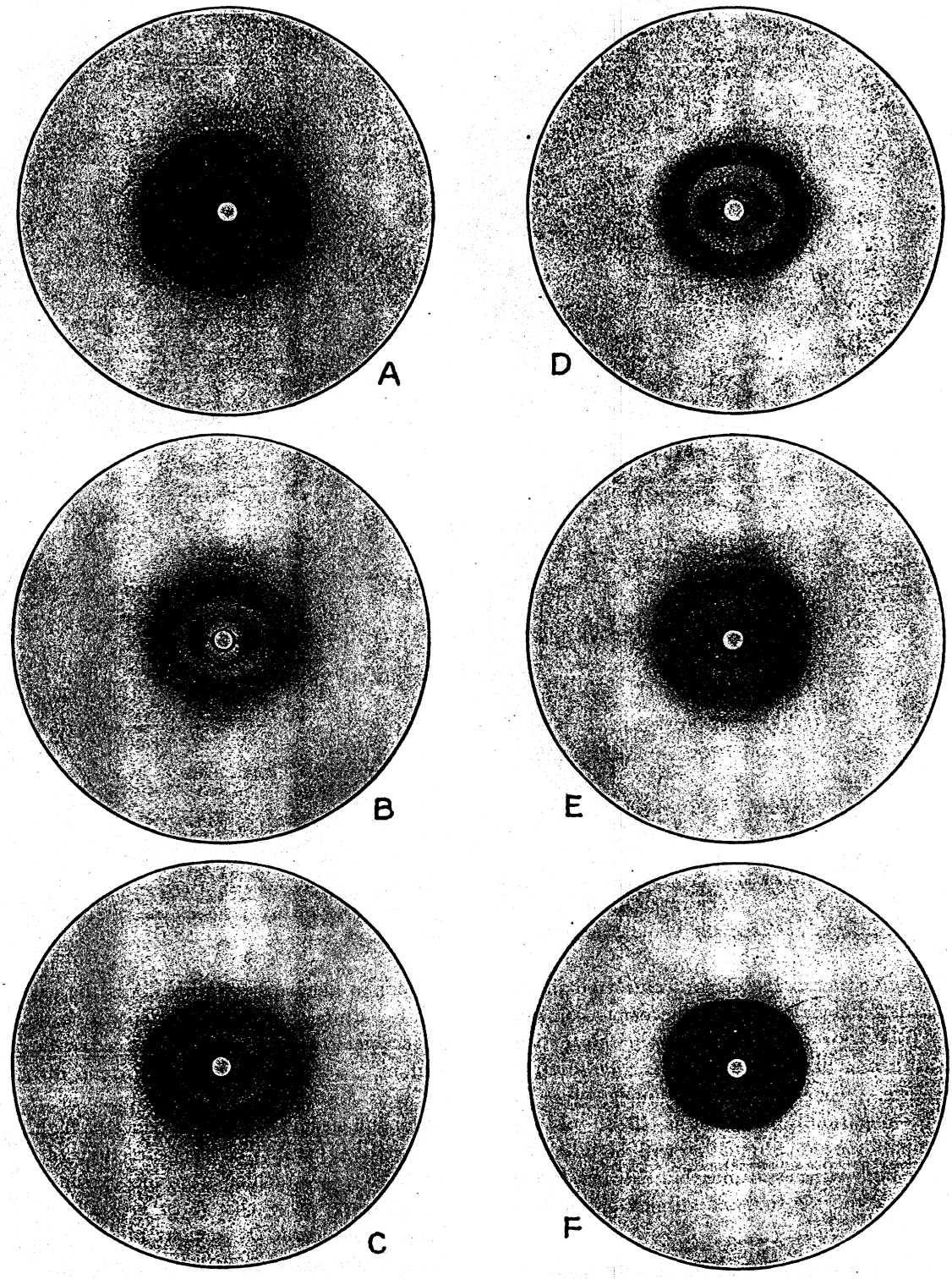

FIG 4. *Diffraction patterns of ovalbumin (A, B, C) and keratin (D, E, F) filaments. (A) Ovalbumin, r.l. = 1; (B) r.l. = 10.5; (C) oriented filament after boiling 8 hours in distilled water; (D) unoriented keratin (horsehair), α-structure; (E) β-structure, r.l. = 1.7; (F) supercontracted, r.l. = 0.7. Ni-filtered Cu radiation; 2.5-cm. specimen to film distance. Fiber axis vertical.*

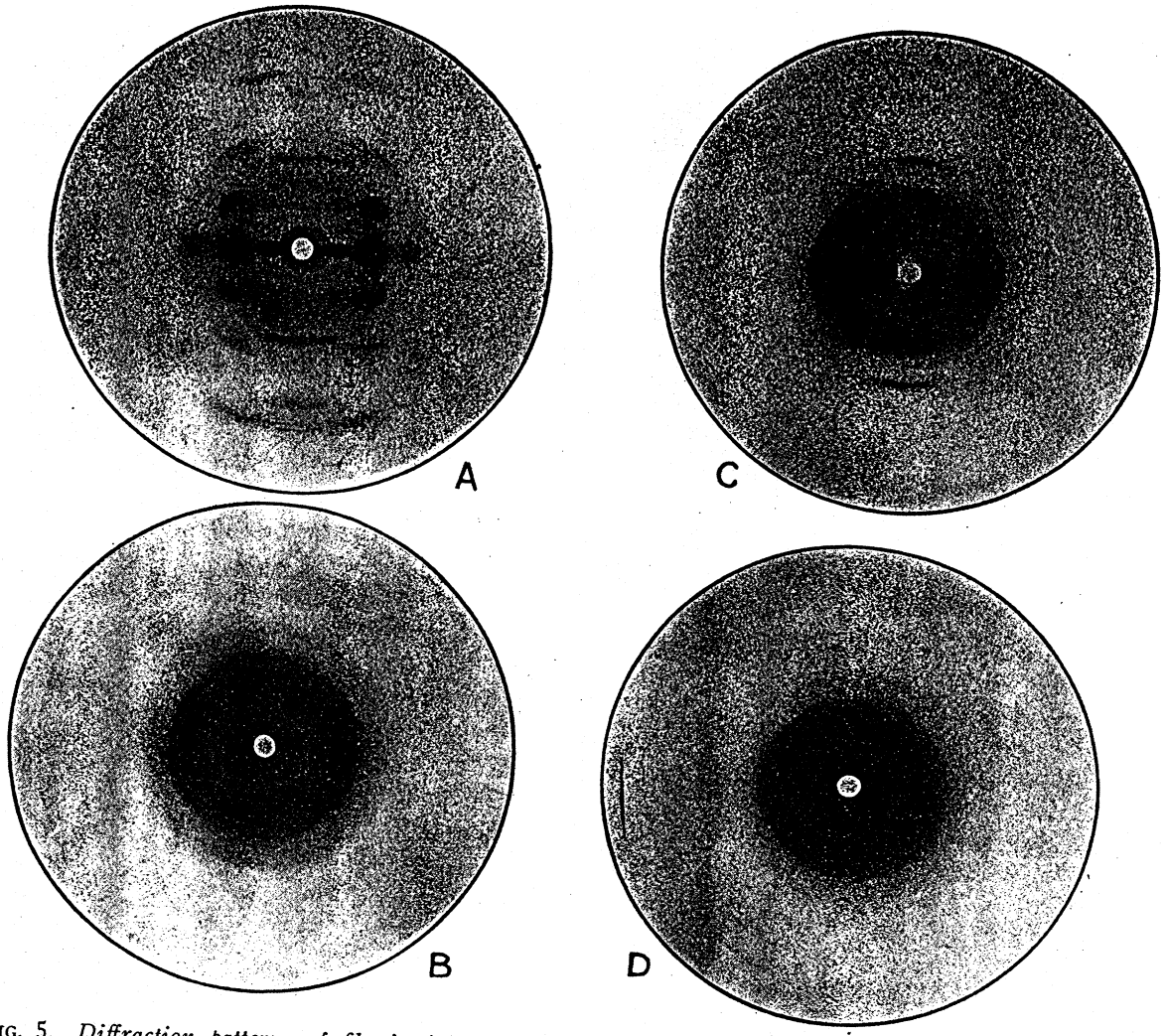

FIG. 5. *Diffraction patterns of fibroin (A, B) and collagen (C, D) filaments. (A) Oriented fibroin, r.l. = 1; (B) contracted fibroin, r.l. = 0.5; (C) oriented collagen, r.l. = 1; (D) contracted collagen, r.l. = 0.5. Ni-filtered Cu radiation, 2.5-cm. specimen to film distance. Fiber axis vertical.*

Fibroin. Neither oriented nor contracted fibroin showed a definite yield point. The elongation (9.1 percent) found for oriented fibroin in this experiment was lower than the values usually given in the literature. Appel [11], for example, gives the range of elongation of fibroin as 13–20 percent. The combination of low elongation with high tenacity produces a high stiffness value, but, because of the limited elongation, the toughness index is relatively low.

Diffraction and Birefringence Observations

Primary indication of changes in configuration and spatial order was by means of x-ray diffraction. Figures 4A, B, and C reproduce diffraction patterns of ovalbumin filaments. Figure 4A is the pattern of a heat-denatured, unstretched preparation (r.l. 1). The sharpness of the rings indicates a fair degree of crystallinity, and the distribution of the diffracting units with respect to the fiber axis is random. Stretching in steam to r.l. 10.5 produced a high degree of orientation parallel to the direction of stretch (Figure 4B), but, judging from the sharpness of the arcs, there was little change in crystallinity. The crystallites or diffracting regions of highly oriented ovalbumin are surprisingly stable toward boiling water. Figure 4C is the pattern of a fiber, originally at r.l. 10.5, that had been boiled 8 hours in distilled water. Although the arcs are lengthened slightly and are somewhat less distinct, it is apparent that the

crystallites have suffered little damage. Previous experience [3] indicates that such treatment with boiling water would result in longitudinal contraction of the fiber of roughly 35 percent and in a corresponding large loss in tensile strength. Both effects must have developed principally in noncrystalline regions.

Figures 4D, E, and F show horsehair keratin in the *alpha-* form (r.l. 1), *beta-* form (r.l. 1.62), and supercontracted form (r.l. 0.71). The practical identity of highly oriented ovalbumin and *beta*-keratin in their crystallite configuration is obvious. Reference to the stretch given the two fibers (r.l. 1.62 and 10.5) illustrates the far greater ease of orientation of the keratins compared with ovalbumin. Lacking the numerous cystine cross-bonds of keratin, molecules and micelles of ovalbumin fibers stretched in steam flow easily and only a minor part of the work of stretching is effective in securing fiber orientation. Supercontraction removed most but not all orientation. In Figure 4F there is slight concentration of intensity in the inner (9.7 Å.) ring in the equatorial direction. The pattern corresponds to ovalbumin stretched in steam to r.l. approximately 1.5. Crystallinity is less, however, for the pattern is more diffuse and the 3.7 Å. (outer) ring is not resolved. No counterpart of the *alpha*-keratin structure has yet been reported for ovalbumin or other globular protein.

Figure 5A is the fiber pattern of fibroin; Figure 5B the pattern for fibroin contracted in 28*M* formic acid to r.l. 0.45. Figure 5C is the diffraction pattern of oriented collagen; Figure 5D is that of the same material contracted to r.l. 0.63 by brief immersion in hot water. As with *beta*-keratin, contraction of both fibroin and collagen greatly diminished the degree of orientation, without changing the structure of the crystallites.

Birefringence also measures molecular orientation, summing effects of both amorphous and crystalline components, and showing particular sensitivity for low and otherwise undetectable degrees of orientation. Table IV gives the birefringence of the six protein fibers with which we have worked. *Beta*-keratin has little higher birefringence than *alpha*-keratin, but its value is much greater than that for supercontracted keratin. While the birefringence of fibroin is considerably reduced on contraction, in both stages of orientation it is uniquely high among protein fibers. Birefringence of collagen is lowered almost 80 percent on contraction; its maximum value is the least of any of the fibers sufficiently oriented to give a well-defined fiber diffraction pattern. Orientation was in no case completely removed by contraction, for positive birefringence was measured in all fibers but unstretched ovalbumin. Residual birefringence in the contracted ovalbumin fiber whose diffraction pattern is shown in Figure 4C was 6.2×10^{-3}. This fiber, of initial r.l. 10.5, shrank ⅓ during 8 hours' boiling.

TABLE IV. Birefringence of Protein Fibers

Fiber	Relative length	Birefringence
Keratin		
Horsehair, *alpha*	1.0	8.6×10^{-3}
Horsehair, *beta*	1.7	10.4
Horsehair, contracted	0.76	2.0
Wool, *alpha*	1.0	7.6
Wool, *beta*	1.5	9.8
Wool, contracted	0.82	3.5
Mohair, *alpha*	1.0	8.2
Mohair, *beta*	1.8	8.6
Fibroin, normal	1.0	73.0
Fibroin, contracted	0.37	33.0
Collagen, oriented	1.0	5.9
Collagen, contracted	0.55	1.2
Ovalbumin *	1.0	0.0
Ovalbumin *	2.0	5.0
Ovalbumin *	6.0	12.0

* Data from reference [3], p. 205.

Discussion

Keratin fibers differ from the others in that they are cellular. In comparing them the assumption was necessarily made that the mechanical and rheological properties measured depend predominantly on intracellular material. Woods [12] had previously shown that the elasticity behavior of a single cortical cell of wool closely approximated that of the complete wool fiber. If there is an effect of cell structure on tensile properties, little evidence for it emerges from this study.

Speakman [5] has attributed supercontraction in the keratins to the breakdown of disulfide cross-links and electrostatic bonds, and permanent set to the breakage of these bonds with subsequent formation of new bonds. If this is correct, then the changes in properties observed on converting keratin to the *beta*- or to the supercontracted form could be due to chemical alteration as well as to changes in the state of orientation. Preliminary analyses for cystine and cysteine [13], however, revealed no significant differences in the amount of these substances in set and supercontracted keratin fibers as compared with the *alpha*-keratin controls. Work on this aspect of the problem is continuing. It should be noted in this connection that Patterson *et al.* [14] found that breaking

the cystine cross-links in wool, by reduction with thioglycolic acid, produced little change in the dry tenacity. It is difficult to judge the effect of the broken electrostatic bonds, since the proportion of these required to be broken in order to bring about supercontraction or permanent set is unknown. Moreover, it is to be expected that most of them would re-form when the stress which caused them to break was removed.

As was stated in the introductory paragraph, among the dozen globular proteins that have been given linear form demonstrable by x-ray diffraction, ovalbumin is outstanding in the extent to which molecular orientation and crystallinity can be produced in it. In the present work it has been shown that maximum orientation raises dry and wet tenacity, but not to the extent that they are comparable with fibroin and collagen; and in achieving maximum tenacity through maximum orientation other important fiber qualities—elongation, flexibility, toughness—are largely sacrificed. Dry tenacity of high-orientation ovalbumin is about the same as that of keratin fibers. Wet tenacity is appreciably lower, but may be made equal by cross-linking with quinone or formaldehyde. Oriented ovalbumin has not, in our experience, shown any tendency to relax into a regularly coiled molecular configuration, and thus lacks the longe-range reversible extensibility that is so usefully characteristic of the keratins. Fibroin and collagen are distinguished from ovalbumin by their flexibility at high orientation. From this difference, particularly, we infer a low effective molecular weight for ovalbumin [3]. Experiments have been begun to measure the effective molecular weight or polymerization degree of the orienting units in this and other globular proteins.

Summary

1. Fibers compared were heat-denatured ovalbumin stretched up to 500 percent in steam but not hardened; keratin (wool, mohair, horsehair) in the *alpha-*, *beta-*, and supercontracted states; fibroin in its normal, oriented form, and after contraction by 8.4*M* HCl; collagen (surgical gut) when oriented and after contraction in hot water.

2. X-ray diffraction patterns demonstrate the near identity of the spatial configuration of *beta*-keratin and highly stretched ovalbumin, and of supercontracted keratin and slightly stretched ovalbumin. Contraction of all the natural fibers is attended by serious loss of orientation, but lateral order or cystallinity in the diffracting units is little affected.

3. Positive birefringence shown by all the fibers is considerably reduced on contraction but does not vanish, demonstrating a fair degree of residual orientation in the contracted fibers. Conversion of *alpha-* to *beta*-keratin produces only a small increase in birefringence.

4. Wet and dry strength increase markedly with molecular orientation. Flexibility, as measured by knot tenacity, decreases.

5. Changes in load-elongation properties of the fibers with orientation are shown. From load-elongation curves, values for elasticity, ultimate elongation, stiffness, and toughness have been tabulated.

6. Tensile properties of *beta*-keratin horsehair and maximally oriented and crystallized ovalbumin are remarkably similar. Tenacity increase in both fibers on maximum orientation is more than offset by unfavorable changes in toughness, stiffness, and elongation.

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